

Seasonal Changes of Triiodothyronine Binding to Piscine Ovarian Nuclei

GAURANGI MAITRA and SAMIR BHATTACHARYA¹

Department of Zoology, Visva-Bharati University
Santiniketan-731235, West Bengal, India

ABSTRACT—The pattern of ¹²⁵I-triiodothyronine (¹²⁵I-T₃) binding to the fairly pure oocytes nuclei preparation and plasma T₃ levels were studied in the seasonally breeding fresh water perch, *Anabas testudineus*. ¹²⁵I-T₃ binding affinity was found to remain fairly constant (K_a varied from 0.11 to 0.12 × 10⁹ M⁻¹) during the four reproductive phases while maximum binding capacity (MBC) altered significantly. The highest value was seen in the prespawning phase (MBC=4.13 p mole/mg DNA) and the lowest in postspawning phase (MBC=3.44 p mole/mg DNA). Plasma T₃ level was in a peak during prespawning (2.9 ng/ml) whereas it was the lowest in postspawning phase fish (0.92 ng/ml). Seasonal indices of plasma T₃ level and MBC suggest an autoregulation of T₃ binding site. Further evidences in support of this were obtained from fish treated with T₃, thiourea and thiouracil; T₃ increased ¹²⁵I-T₃ binding while antithyroid drugs decreased it significantly.

INTRODUCTION

Several reports support the involvement of thyroid in vertebrate reproduction including fishes [1-6]. Whether thyroid influences gonadal activity directly or indirectly is still unclear. In seasonally breeding lower vertebrates, such as fish, the gonadal cycle coincides with the thyroid cycle [7-9]. Thyroid influence on the piscine ovary has been viewed by some as an indirect one and suggested that thyroid hormone increased ovarian response to gonadotropin [10-12], while others indicated an individual effect of thyroid hormone on piscine ovary as ovarian metabolic activity considerably increased in *in vitro* [13-14]. Recently, we have reported high affinity, low capacity thyroid hormone binding sites in the nuclei of perch ovary [15]. This indicates that thyroid hormone has distinct physiological relevance in relation to reproduction. Since this perch is a seasonally breeding fish, it will be interesting to note the profile of triiodothyronine (T₃) binding to ovarian nuclei in relation to the seasonal reproduc-

tive cycle. The present report deals with the T₃ binding patterns in perch ovarian nuclei at four distinct reproductive phases and how this information leads to the revelation of autoregulation of ovarian thyroid hormone receptor.

MATERIALS AND METHODS

Anabas testudineus is a freshwater perch occurring commonly in India. Its reproductive cycle can be divided into four distinct phases: i) preparatory (February - April) ii) prespawning (May - June) iii) spawning (July - early September) and iv) postspawning (late September - January). Adult female perch (body wt. 20-25 g; length 10-12 cm) were collected at different reproductive phases from local ponds. Method used for the collection of blood and ovarian tissue were similar to a paper reported earlier [9]. Blood was collected from the caudal vein with 1 ml syringe (24 gauge needle), plasma was separated and stored at -20°C. Plasma T₃ level was determined by specific radioimmunoassay (RIA) after our earlier report [9].

Ovaries collected from perch belonging to different reproductive phases, and subjected to homogenization followed by isolation of nuclei [16] for its *in vitro* binding incubation. Procedures

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¹ To whom reprint requests should be addressed.

followed for isolating the nuclei from perch ovary, its quantitation (as reflected by DNA concentration) and ^{125}I - T_3 binding assay were similar to our earlier report [15]. In the binding assay $80\text{ }\mu\text{g}$ DNA was incubated with 1.6 p mole of ^{125}I - T_3 (sp. activity $180.65\text{ }\mu\text{Ci}/\mu\text{g}$, Bhabha Atomic Research Centre, India) in $500\text{ }\mu\text{l}$ binding incubation medium, with or without 400 p mole of cold T_3 . Specific binding was calculated by subtracting nonspecific binding and expressed as a percentage of total binding.

Adult female perch used for T_3 and thiourea treatment were acclimatized for 7 days, fed and maintained in a manner as described before [9]. Preparatory stage fish were selected for this experi-

ment as endogenous T_3 level was found to be low and thus response to exogenous T_3 by the ovary was expected to be better. T_3 , thiouracil and thiourea were injected separately and intramuscularly on the dorsal peduncle ($100\text{ ng}/100\text{ g body wt}$ and $2\text{ }\mu\text{g}/100\text{ g body wt}$ respectively) for 7 days. Control group received saline (vehicle) in equal volume. Ovaries thereafter collected from treated and control groups, processed for obtaining nuclei receptor material and subjected to binding assay.

For *in vitro* T_3 treatment of perch ovarian tissue (from prespawning stage fish), oocytes were separated and incubated by following the procedure described earlier [17]. Incubations were made with T_3 ($100\text{ ng}/\text{incubation}$) or 0.6% saline for 6 hr and

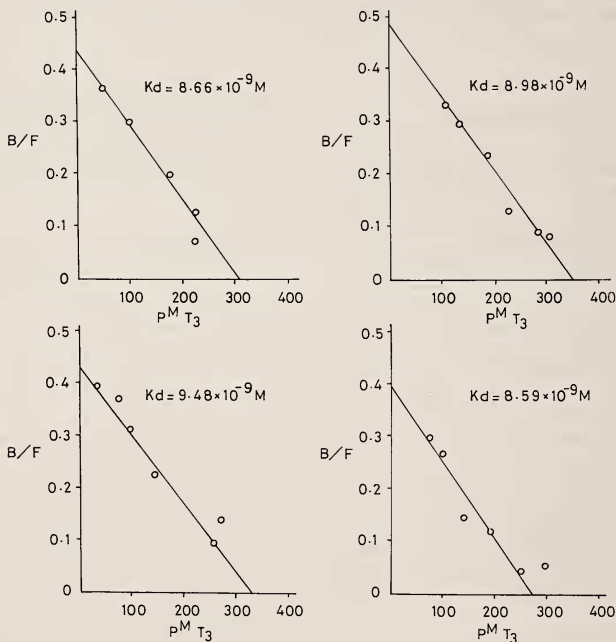


FIG. 1. Scatchard analysis of T_3 binding to ovarian nuclei in different seasons. Each point is the mean of 6 tests.

terminated by transferring them to an ice bed. The oocytes were immediately processed for nuclei isolation, extraction and binding assay. Student's *t*-test [18] was applied to analyse the data.

RESULTS

T₃ binding to perch ovarian nuclei of different reproductive phases was assessed by incubating 80 µg of DNA with increasing concentrations of radiolabeled T₃ (0.41 to 3.2 p mole) keeping other conditions constant. Scatchard analysis for the four reproductive phases shows that high affinity, low capacity T₃ binding sites for ovarian nuclei were clearly detectable in all stages. The K_a for different phases did not vary significantly (between 0.11 to 0.12 × 10⁹ M⁻¹, Fig. 1).

Maximum binding capacity (MBC) on the other hand, varied remarkably in different seasons (Table 1). MBC was the highest during prespawning and spawning phases, which was significantly reduced in the postspawning stage ($p < 0.02$ in comparison to prespawning) and this lower MBC continued till the preparatory stage. Table 1 also shows that the peak MBC values coincided with the peak seasonal plasma T₃ levels. This indicates that the amount of ovarian nuclear T₃ binding sites may be dependent on its own ligand level in the circulation. To investigate such a possibility, binding of T₃ to ovarian tissue of T₃ and thiourea treated fish was compared. Figure 2 demonstrates the results of this experiment. Treatment of T₃ increased its specific binding significantly ($p < 0.001$) while treatment with thyroid inhibitors, thiourea and thiouracil, decreased T₃ level remarkably ($p < 0.001$). To confirm this, oocytes were incubated *in vitro* with T₃ and nuclei isolated from them showed a comparable increase in T₃ binding over the control (Fig. 3).

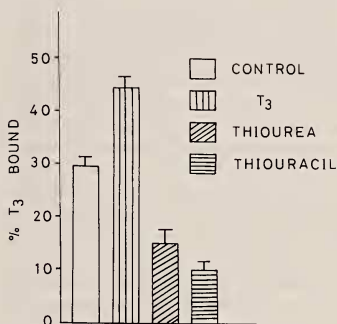


FIG. 2. Effect of T₃, thiourea and thiouracil on T₃ binding to perch ovarian nuclei. Vertical bar in each case represents mean for 3 tests ($\pm$ SE).

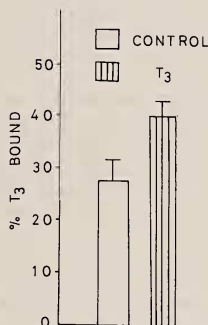


FIG. 3. *In vitro* incubation of perch oocytes with T₃. Vertical bar represents mean of 4 tests ($\pm$ SE).

TABLE 1. Plasma T₃ levels and MBC in different seasons

Season	Plasma T ₃ level ^a ng/ml	Maximum binding ^b capacity p mol/mg DNA
Preparatory	1.70 $\pm$ 0.04	3.813 $\pm$ 0.91
Prespawning	2.90 $\pm$ 0.28	4.313 $\pm$ 0.71
Spawning	1.05 $\pm$ 0.02	4.125 $\pm$ 0.24
Postspawning	0.92 $\pm$ 0.02	3.440 $\pm$ 0.08

^{a,b} Mean $\pm$ SE of 6 determinations.

DISCUSSION

The present work is an attempt to elucidate the seasonal profile of ovarian thyroid hormone receptor in a seasonally breeding teleost. Freshwater perch, *A. testudineus*, breeds once a year and that is during the monsoon or rainy season in India. To explain the physiological relevance of ovarian thyroid hormone receptor [15], a study in relation to reproductive cycle, which is categorised into four stages, appears to be important. Scatchard analysis of T_3 binding to the ovarian nuclei of perch for 4 reproductive phases indicates that affinity for binding sites remained almost unaltered as K_a values varied between 0.11 to $0.12 \times 10^9 M^{-1}$. This appears to be a little surprising since the size and weight of the perch ovary varied strikingly in different reproductive phases. But alteration of MBC in different reproductive phases correlates ovarian size and weight. MBC was found to be highest in prespawning fish ovarian nuclei. There was a significant drop in MBC in the ovarian nuclei of postspawning fish and a similar MBC value was obtained for preparatory stage fish. All these observations indicate that affinity of T_3 for the ovarian binding site does not alter in different phases of reproductive cycle while the amount of receptor occupancy has distinct variation.

Seasonal profile of plasma T_3 level in perch coincides with peak of MBC value, both are the highest in prespawning phase. Report from this laboratory indicates that reproductive metabolism of this perch was the highest in the prespawning, while it decreased, although not significantly, in the spawning phase, followed by a significant fall in the postspawning phase [19]. Plasma level and MBC of T_3 followed the same pattern. It has been suggested that temperature plays a important role in elevating plasma level of thyroid hormone in fish [20], whereas on the photoperiod there is no such report in fish. There is a considerable rise of temperature from preparatory to prespawning and in fact the highest temperature is recorded in May and June in this area of India. This particular environmental factor is also found to be very important in this perch, as keeping them at $30-35^\circ C$ regime not only enhanced thyroid hormone level but also induced gonadal maturation in pre-

paratory stage fish (data not shown). Hence it may be presumed that an increase in environmental temperature resulted higher plasma T_3 level and this in turn caused higher MBC in the prespawning fish.

To assess such possibility, perch of the preparatory stage were treated with T_3 , thiourea and thiouracil for 7 days. T_3 significantly increased the binding of $^{125}I-T_3$ while there was a remarkable drop in T_3 binding by thiourea and thiouracil in comparison with control. Treatment by thiourea resulted significant fall of thyroid hormone level in plasma (data not shown). Since *in vivo* experiments face a question of interference or mediation by some endogenous factor, an *in vitro* incubation of oocytes with T_3 was performed. Increase of $^{125}I-T_3$ binding to ovarian nuclei was again clear.

Evidences so far obtained from seasonal and experimental data provide two important information—1) a direct relation of thyroid with seasonal reproduction of perch 2) thyroid hormone regulates T_3 binding sites in the ovarian nuclei. These two information are relevant in assessing the thyroid function related to reproduction of teleostean fishes.

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REFERENCES

- 1 Ball, J. N. (1960) Reproduction in female bony fishes. Symp. Zool. Soc. London, 1: 105-135.
- 2 Fontaine, Y. A. (1975) Hormones in fishes. In "Biochemical and Biophysical Perspectives in Marine Biology". Ed. by D. C. Malins and J. R. Sargents, Academic Press, Orlando, Vol. 2, pp. 139-212.
- 3 Bona-Gallo, A., Licht, P., Mackenzie, D. S. and Lofts, B. (1980) Annual cycles in levels of pituitary and plasma gonadotropin, gonadal steroids and thyroid activity in the Chinese cobra (*Naja naja*). Gen. Comp. Endocrinol., 42: 477-493.
- 4 Ingbar, S. H. and Woebber, K. A. (1980) The Thyroid Gland. In "Textbook of Endocrinology". Ed. by R. H. Williams, Igakushoin/Saunders International Edition, Philadelphia/Tokyo, pp. 117-243.
- 5 Bhattacharya, S., Sen, S. and Deb, S. (1985) Hor-

- monal regulation of ovarian 17 β -hydroxy-steroid dehydrogenase in teleost. In "Current Trends in Comparative Endocrinology". Ed. by B. Lofis and W. N. Holmes, Hong Kong Univ. Press, Hong Kong, Vol. II, pp. 237-238.
- 6 Tasaki, Y., Inoue, M. and Ishii, S. (1986) Annual cycle of plasma thyroid hormone levels in the toad, *Bufo japonicus*. Gen. Comp. Endocrinol., **62**: 404-410.
- 7 Ichikawa, M., Mori, T., Kawashima, S., Ueda, K. and Shirahata, S. (1974) Histological changes in the thyroid and internal tissue of kokanee (*Oncorhynchus nerka*) during sexual maturation and spawning. J. Fac. Sci. Univ. Tokyo, **4**: 175-182.
- 8 White, B. and Henderson, N. E. (1977) Annual variations in the circulating levels of thyroid hormones in the brook trout, *Salvelinus fontinalis*, as measured by radioimmunoassay. Canad. J. Zool. **55**: 475-481.
- 9 Chakraborti, P. and Bhattacharya, S. (1984) Plasma thyroxine levels in freshwater perch: Influence of season, gonadotropins and gonadal hormones. Gen. Comp. Endocrinol., **53**: 179-186.
- 10 Hurlburt, M. E. (1977) Role of thyroid gland in ovarian maturation of the goldfish, *Carassius auratus* L. Canad. J. Zool., **55**: 1906-1913.
- 11 Dettlaff, T. A. and Davydova, S. I. (1974) Effect of triiodothyronine on the ripening of oocytes in stellate sturgeon after action of low temperatures and reservation of females. Soc. J. Dev. Biol., **5**: 402-409.
- 12 Dettlaff, T. A. and Davydova, S. I. (1979) Differential sensitivity of cells of follicular epithelium and oocytes in the stellate sturgeon to unfavourable conditions and correlating influence of triiodothyronine. Gen. Comp. Endocrinol., **39**: 229-232.
- 13 Sen, S. and Bhattacharya, S. (1982) Hormonal influence on perch ovarian 17 β -hydroxysteroid dehydrogenase activity in *in vitro* system. Indian J. Exp. Biol., **20**: 664-667.
- 14 Sen, S. and Bhattacharya, S. (1981) Role of thyroxine and gonadotropin on the mobilization of ovarian cholesterol in a teleost, *Anabas testudineus* (Bloch). Indian J. Exp. Biol., **19**: 408-412.
- 15 Chakraborti, P., Maitra, G. and Bhattacharya, S. (1986) Binding of thyroid hormone to isolated ovarian nuclei from a freshwater perch, *Anabas testudineus*. Gen. Comp. Endocrinol., **62**: 239-246.
- 16 Jackson, V. and Chalkey, R. (1974) Separation of newly synthesized nucleohistone by equilibrium centrifugation in calcium chloride. Biochemistry, **13**: 3952-3956.
- 17 Deb, S., Jamaluddin, Md., Bhadra, R., Bhattacharya, S. and Datta, A. G. (1985) Bioassay of fish gonadotropin by ovarian mitochondrial cholesterol depletion. Gen. Comp. Endocrinol., **57**: 491-497.
- 18 Snedecor, G. W. and Cochran, W. G. (1971) "Statistical Methods", 6th Ed. Iowa State University Press, Ames.
- 19 Deb, S. and Bhattacharya, S. (1986) Circulatory cholesterol as an important source of substrate for piscine ovarian steroidogenesis. Indian J. Exp. Biol., **24**: 71-76.
- 20 Leloup, J. and Deluze, A. (1985) Environmental effects of temperature and salinity on thyroid function in teleost fishes. In "Endocrine and the Environment". Ed. by B. K. Follett, S. Ishii and A. Chandra, Japan Sci. Soc. Press, Tokyo/Springer-Verlag, Berlin, pp. 23-32.